https://doi.org/10.17221/49/2021-CJGPB

Comparative analysis of late blight resistance *R* genes and their coding proteins in some potato genotypes

Heba Amin Mahfouze, Osama Ezzat El-Sayed*

Genetics and Cytology Department, National Research Centre, Dokki, Giza, Egypt

**Corresponding author: hebaamn@yahoo.com*

Citation: Mahfouze H.A., El-Sayed O.E. (2022): Comparative analysis of late blight resistance *R* genes and their coding proteins in some potato genotypes. Czech J. Genet. Plant Breed., 58: 10–20.

Abstract: Late blight (LB) disease can cause potato yield losses in both Egypt and the world. Therefore, the structural analysis of resistance (*R*) genes responsible for LB resistance will help in understanding their functions. This work aimed to identify the variations between the dominant and recessive alleles of two genes, *R3a* and *R8* at the nucleotide and amino acid levels in five potato genotypes. Two genes of *R3a* and *R8* representing the broad-spectrum LB resistance were amplified by specific primers, which gave one amplicon of 194 and 220 bp of each gene, respectively. Two fragments were sequenced after purification using an ABI 3730xl System DNA Sequencer. The DNA sequence alignments of two genes, *R3a* and *R8*, were determined among five selected potato genotypes. The percentage of genetic similarity of the nucleotide sequences of the *R3a* and *R8* genes ranged between (82–83%) and (86–87%), respectively, in comparison to the reference sequences in the nucleotide BLAST. We report on the existence of positional differences in the nucleotide sequences, and base-pair substitutions of two fragments, resulting in amino acid changes between the resistant and susceptible potato genotypes. On the other hand, the highest total number of base-pair substitutions was recorded as 16 in the recessive allele *r8* of the varieties Bellini and Cara. However, the lowest number was recorded as four in the dominant allele *R3a* of the variety Cara. The dendrograms of the five potato genotypes were made up of phylogenetically different clusters, separate from all the other named potato accessions of the two genes. The results of this study will create a solid base for the further understanding of the mechanism of plant-pathogen interactions and supply a theoretical reference for durable resistance to late blight diseases in the potato.

Keywords: fungus resistance; genetic polymorphism; nucleotide sequencing; *R3a; R8; Solanum tuberosum* L.

The potato (*Solanum tuberosum* L.) is the third most common food crop in the world after wheat and rice (Birch et al. 2012). The potato crop is severely damaged by late blight (LB) disease caused by the oomycete *Phytophthora infestans* (Lenman et al. 2016).

Genetic resistance is considered the mainstay to control the *P. infestans* disease as an alternative way to using fungicides. The application of chemical fungicides is costly to farmers and also raises environmental pollution in both developed and developing countries. Besides, the fungus could mutate itself to be resistant to particular fungicides used to control the LB pathogen. In *S. tuberosum* L., most of the *R* loci belong to complex *R*-gene clusters with closely linked paralogs (Gebhardt & Valkonen 2001). In gene-for-gene theory, the host R proteins can be recognised by the microorganism *Avr* or effector proteins either directly by the R-Avr protein-protein interaction or indirectly by changes in the plant host proteins, known as guardee proteins in the guard hypothesis (Petit-Houdenot & Fudal 2017). Some *R* loci were cloned, identified and showed that nucleotide-binding site-leucine-rich repeat (NBS-LRR) loci form the biggest plant host resistance locus family, repre-

Supported by Project No. 12020111 funded by National Research Centre, Dokki, Giza, Egypt.

https://doi.org/10.17221/49/2021-CJGPB

senting about 80% of more than 140 cloned *R* loci (Shao et al. 2016). However, the search for *R* locus variants supplying plant cultivars with resistance to a specific microorganism or novel microorganism isolates is still difficult and time-consuming. In this field, novel reports have been developed on the principle of genome-wide resequencing and comparison with reference genomes that have shown a number of NBS-LRR candidate loci (Kochetov et al. 2017). Comparison of syntenic genomic sites of closely related plant species containing NBS-LRR loci was indicated to be a hopeful method to determine candidate resistance loci in the genomes of different plant crops (Quirin et al. 2012).

To date, more than twenty resistance (*R*) loci have been cloned from wild *Solanum* sp. involving the LB resistance loci *R1*, *R3a* and *R3b* (Vossen et al. 2016; Jiang et al. 2018). The *R3* gene of *S. tuberosum* L. refers to the full resistance to avirulent races of *P. infestans* and consists of two functional loci, *R3a* and *R3b*, which are 0.4 centimorgans (cM) apart. A recombination event between the two loci allowed their separation, and the successive identification of the recombinant plants indicated that the two loci have various pathogen race-recognition specificities (Huang et al. 2004). The *R3b* locus composed of one exon has one open-reading frame (ORF) of 3 855 nucleotides, which encodes to 1 284 amino acids. The *R3b* locus structure is identical to *R3a*, encoding a putative a coiled-coil CC-NBS-LRR protein. Both the *R3a* and *R3b* loci scored 82 and 73% similarity at the nucleotide and amino acid levels, respectively (Huang et al. 2005). Further analysis indicated that the *R3a* and *R3b* loci encoded 29 and 28 LRRs, respectively and each encoded two unique LRRs (Li et al. 2011). Furthermore, the wide ranging LB resistance locus *R8* from *S. demissum* is mapped at chromosome 9 and encodes to a CC-NBS-LRR protein as reported by Vossen et al. (2016).

The purpose of the present research is to characterise, compare, and analyse the nucleotide sequences and the putative proteins of the dominant and recessive alleles of two *R* genes, *R3a* and *R8*, in five potato genotypes resistant and susceptible to LB disease, based on genetic polymorphism and base-pair substitution.

MATERIAL AND METHODS

Plant material. Four potato varieties Jelly, Cara, Bellini and Annabelle were collected from the brown rot project, Dokki, Giza, Egypt, as well as the wild genotype *S. demissum* 1977 supported by the Centre for Genetic Resources, the Netherlands (http://www.wur.nl). In our previous study, we evaluated the five potato genotypes for LB resistance. It was clearly shown that one of the five genotypes was highly resistant (Jelly), one was resistant (*S. demissum* 1977), one was moderately resistant (Cara), and two were moderately susceptible (Bellini and Annabelle) (Mahfouze et al. 2021). Besides, two markers: the cleaved amplified polymorphic sequence (CAPS) and sequence characterised amplified region (SCAR) linked to the *R3a* and *R8* genes were applied to determine the dominant and recessive alleles in four commercial potato varieties [Jelly (*R3a*/*r8*) Cara (*R3a*/*r8*), Bellini (*R3a/r8*), and Annabelle (*r3a/r8*)] and one wild *S. demissum* (*r3a/R8*)] (unpublished data).

Design specific primers for two *R* genes in potato. Two different *S. tuberosum* LB resistance gene sequences; *R3a* and *R8* of accessions AY849382 and KU530153, respectively obtained from the NCBI GenBank were aligned using the MultAlin Fasta format. Two primers for each of the two *R3a* and *R8* genes were designed from the very similar sequences within the consensus as shown in Table 1.

DNA extraction and PCR amplification. The total genomic DNA was extracted from the fresh mature potato leaves cultured in a greenhouse, using a DNeasy plant mini-prep kit (Qiagen, CA).

A polymerase chain reaction (PCR) amplification was performed in a thermal cycler (Biometra, Biomedizinische Analytik GmbH) in a total volume of 25 µL containing 50 ng DNA, 1 mM of each primer, 200 mM dNTPs, 1.5 mM $MgCl_2$ and 0.5 U *Taq* DNA polymerase (GoTaq® DNA Polymerase, Promega, USA). The PCR test was performed under the following conditions: 94 °C at 4 min and then 35 cycles of 94 °C at 1 min, 50 °C at 1 min and 72 °C at 1 min and a final extension step at 72 °C for 5 min.

All the PCR products were electrophoresed on 1% agarose gel electrophoresis in a 1× TBE buffer

Table 1. Forward and reverse primer sequences used for the amplification of the two *R* resistance genes

Genes	Forward & reverse primers	Primer sequences (5'-3')	Size (bp)
R3a/r3a	F	Tgctcggtcttcagattgtg	194
	R	Ttgctggttgcttgtttctg	
R8/r8	F	Gtgggatctcctcaaggtca	220
	R	Tccttcattgcggaactacc	

https://doi.org/10.17221/49/2021-CJGPB

(89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3). The genomic DNA was stained with a RedSafe Nucleic Acid Staining Solution (1/20 000) (iNtRON Biotechnology, Inc. South Korea) and was visualised and photographed with a Gel-Documentation system (Bio-Rad Laboratories, Inc., CA, USA). The size of each fragment was estimated with reference to a size marker of a 100 bp DNA ladder (BioRoN, Germany).

Purification, sequencing and analysis of the two *R* genes. The PCR products of two *R3a* and *R8* fragments were purified with a QIA quick PCR Purification Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions and the DNA was eluted in 20 µL of sterile ddH_2O. The two amplified fragments were sequenced on a Capillary Electrophoresis Sequencing (CES) automation system (ABI 3730xl System DNA Sequencer, Macrogen, South Korea).

Alignment and phylogenetic tree. The sequences were compared with the sequences of the representatives of the most related *S. tuberosum* accessions deposited in the GenBank and sequencing-genome databases by using the BLAST search (http://www.ncbi.nih.gov/blast). The analysis was performed using the Geneious Pro program (Ver. 4.5.4). A phylogenetic tree was constructed by the neighbour-joining method and a dendrogram was constructed using multiple alignments of the two *R* genes from the potato clones.

RESULTS

PCR amplification of *R* genes in resistant and susceptible potato genotypes. The PCR amplification of the resistant potato varieties (Jelly, Cara and *S. demissum* 1977) and the susceptible ones (Annabelle and Bellini) scored two fragments with expected sizes of 194 and 220 bp that represented the *R3a* and *R8* genes, respectively, using two specific primers (Figure 1).

Sequence analysis of two *R* genes in the resistant and susceptible potato genotypes. The partial nucleotide sequences of the *R3a* (194 bp) gene in each of the five tested potato genotypes were aligned and compared with a total of 100 partial nucleotide sequences of the *R* genes in the GenBank, using the BLAST search (Table 2, Figure 2). The BLAST sequence analysis showed that the *R3a* gene under study had an identity that ranged from 82 to 83% with the *R3a* genes recorded in GenBank, whereas the total score and coverage percentage revealed 100 bits and from 82 to 97%, respectively, as are shown in Table 2 and Figure 2. For *R8*, the partial nucleotide sequence of the *R8* locus (220 bp) was aligned and compared with 77 *R8* resistance genes from the different potato accessions published in the GenBank. The nucleotide-nucleotide BLAST matched an 87% identity with Cara, Bellini and Annabelle, while Jelly and *S. demissum* shared 86% sequence homology. The coverage percentage revealed a range from 26 to 93% (Table 2, Figure 3). Therefore, multiple alignments of the two genes under study of the five potato genotypes revealed positional differences in the nucleotide sequences and base-pair substitutions compared with the potato accessions in the National Center for Biotechnology Information (NCBI) (Figures 2, 3).

Nucleotide statistics of two *R* genes in the resistant and susceptible genotypes. The nucleotide statistics of the two fragments in the five potato genotypes that are resistant and susceptible to LB are presented in Table 3. The average molecular weight of the dsDNA (115.22 kDa) was two-fold that of the ssDNA (57.44 kDa) in both the resistant and susceptible potato genotypes. On the other hand, we compared the base sequences of the dominant alleles (*R3a* and *R8*) and recessive ones (*r3a* and *r8*) in the five potato genotypes where little variations (from 1 to 6 frequencies) were observed, and the percentages of the four nucleotides, A, T, C, and G are shown in Table 3.

Positional differences of the two *R* genes in the resistant and susceptible genotypes. A total number of positional differences and base-pair substitutions between the dominant and recessive alleles of the two loci (*R3a/*r3a and *R8/r8*) in the five potato genotypes were identified. A total number of 115 hits were distributed as 78 transversions (A/C, T/A, C/G, or G/T) and 37 transitions (A/G or T/C),

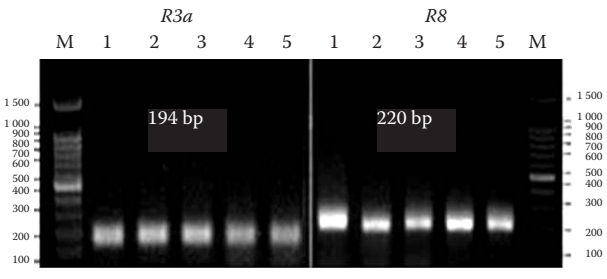

Figure 1. PCR amplified products of the *R3a* and *R8* genes of the five potato genotypes using two designed primers with expected sizes 194 and 220 bp
M – 100 bp DNA ladder, 1 – Jelly; 2 – Cara; 3 – Bellini; 4 – Annabelle; 5 – *S. demissum*

https://doi.org/10.17221/49/2021-CJGPB

Table 2. Blast of the *R3a* and *R8* genes' identity of the five potato genotypes with the corresponding NCBI accession sequences

Genes	Accessions	Descriptions of *R3a* and *R8* genes	Identity % of five potato genotypes				
			highly resistant Jelly (*R3a/r8*)	moderately resistant Cara (*R3a/r8*)	moderately susceptible		resistant *S. demissum* (*r3a/R8*)
					Bellini (*R3a/r8*)	Annabelle (*r3a/r8*)	
R3a	EF613539	*S. demissum* isolate 48G14 disease resistance protein R3a-like protein gene	87	83	82	86	86
	EF613528	*S. demissum* isolate 26N3 disease resistance protein R3a-like protein gene	87	83	82	86	86
	EF613537	*S. demissum* isolate 439G3a disease resistance protein R3a-like protein gene	85	81	80	86	85
	AY849382	*S. demissum* potato late blight resistance protein R3a gene	81	83	82	81	81
	EF613526	*S. demissum* isolate 184J15 disease resistance protein R3a-like protein gene	81	83	82	82	82
	EF613536	*S. demissum* isolate 380L15 disease resistance protein R3a-like protein pseudogene	81	82	82	81	82
	EF613529	*S. demissum* isolate 26O9a disease resistance protein R3a-like protein pseudogene	81	82	82	81	82
	EF613527	*S. demissum* isolate 195A19 disease resistance protein R3a-like protein pseudogene	81	81	80	81	81
	EF613543	*S. demissum* isolate 568A14e disease resistance protein R3a-like protein pseudogene	88	84	–	–	87
	EF613540	*S. demissum* isolate 568A14a disease resistance protein R3a-like protein gene	83	–	83	–	84
	EF613550	*S. demissum* isolate 83C11 disease resistance protein R3a-like protein pseudogene	–	80	79	79	–
	N = 100, query cover: 82–97%						
	average		83	82	82	83	83
R8	KU530153	*S. demissum R8* gene cluster	99	97	98	93	98
	XM_015231221	*S. pennellii* putative late blight resistance protein homolog R1A-3 (LOC107029777)	93	91	93	89	91
	XM_027911991	*S. pennellii* putative late blight resistance protein homolog R1A-3 (LOC107030630)	82	82	82	85	81
	XM_015305029	*S. tuberosum* putative late blight resistance protein homolog R1B-13 (LOC107058848)	95	95	93	93	95
	XM_016640085	*N. tabacum putative* late blight resistance protein homolog R1A-3 (LOC107814641)	85	85	84	83	85
	XM_016648211	*N. tabacum putative* late blight resistance protein homolog R1A-3 (LOC107821761)	85	83	84	83	85
	XM_009805137	*N. sylvestris* putative late blight resistance protein homolog R1A-3 (LOC104248810)	85	85	84	83	85
	XM_009779076	*N. sylvestris* putative late blight resistance protein homolog R1A-3 (LOC104226957)	84	83	84	83	84
	XM_019215571	*S. lycopersicum* putative late blight resistance protein homolog R1A-3 (LOC101256122)	83	82	83	85	82
	XM_016710809	*C. annuum* putative late blight resistance protein homolog R1A-3 (LOC107864451)	77	86	85	88	78
	XM_016710811	*C. annuum* putative late blight resistance protein homolog R1A-3 (LOC107864451)	77	86	85	88	78
	N = 77, query cover: 26–93%						
	average		86	87	87	87	86

https://doi.org/10.17221/49/2021-CJGPB

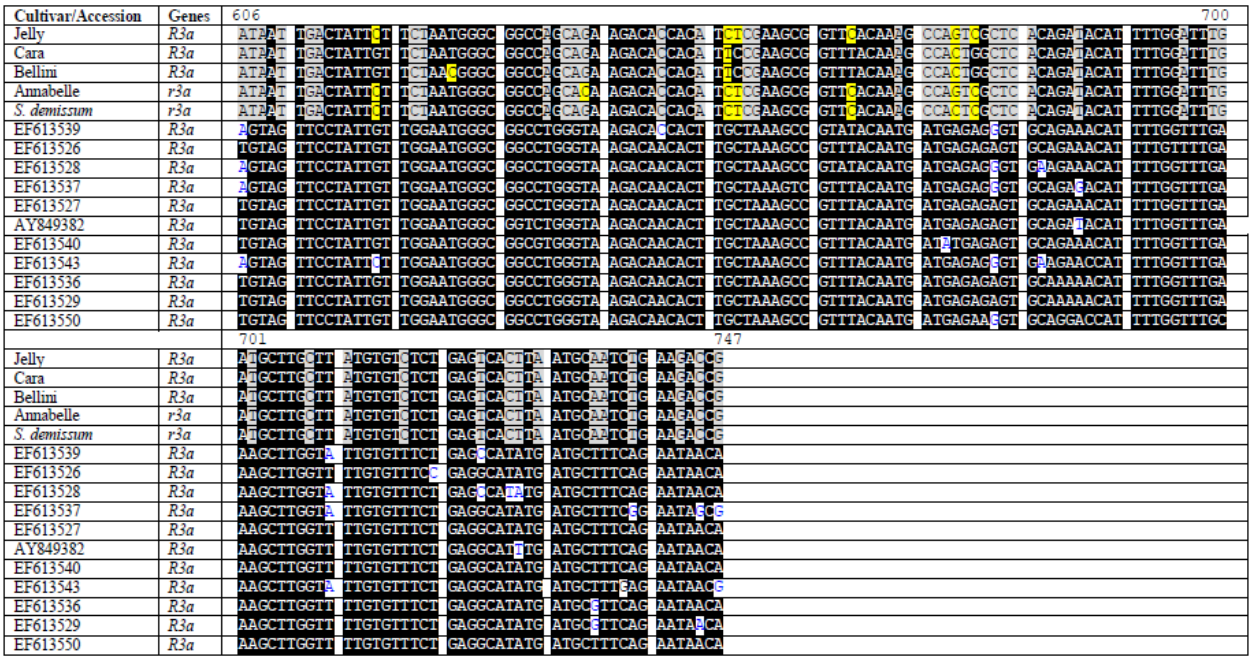

Figure 2. Sequence alignment of the *R3a* and *r3a* genes of the five potato genotypes, compared with the other accessions that exist in the NCBI GenBank

which are shown in Table 4. The highest number of base-pair substitutions was shown in the *R8/r8* genes of the five resistant and susceptible genotypes to LB (75 bp). However, the *R3a/r3a* genes displayed 40 positional exchanges and base-pair substitutions between the dominant and recessive alleles in the five potato clones. The ratio of the transversion was higher than the transition in both genes. The highest number of base-pair substitutions was sixteen, resulting in a nucleotide substitution from T to A, followed by thirteen base-pair substitutions of A→T and T→C. In contrast, the lowest number of base-pair substitutions was two substitutions (C→G). A single base exchange or a mixed base (more than one nucleotide determined at a single position) is considered as a mutation in the gene (Table 4).

Genetic variations and base-pair substitutions of two *R* genes in the potato genotypes. The genome sequence alignment analysis revealed several nucleotide transitions and transversions between the

Table 3. Nucleotide statistics of the two *R* genes in the five potato genotypes

Genes	Varieties	Molecular weight (kDa)		A		C		G		T		GC	
		ssDNA	dsDNA	freq	%	freq	%	freq	%	freq	%	freq	%
R3a	Jelly	54.406	109.356	47	26.6	44	24.9	32	18.1	54	30.5	76	43
R3a	Cara	54.506	109.352	47	26.6	39	22.0	33	18.6	58	32.8	72	41
R3a	Bellini	54.408	109.352	45	25.4	41	23.2	31	17.5	60	33.9	72	41
r3a	Annabelle	54.401	109.351	47	26.6	41	23.2	30	16.9	59	33.3	71	40
r3a	*S. demissum*	54.308	109.356	45	25.4	46	26.0	30	16.9	56	31.6	76	43
r8	Jelly	60.541	121.096	57	29.1	43	21.9	42	21.4	54	27.6	85	43
r8	Cara	60.405	121.095	58	29.6	46	23.5	38	19.4	54	27.6	84	43
r8	Annabelle	60.479	121.096	59	30.1	45	23.0	40	20.4	52	26.5	85	43
r8	Bellini	60.421	121.096	57	29.1	46	23.5	39	19.9	54	27.6	85	43
R8	*S. demissum*	60.558	121.094	60	30.6	42	21.4	41	20.9	53	27.0	83	42
Average		57.44	115.22	52.2	27.91	43.3	23.26	35.6	19	55.4	29.84	78.9	42.2

https://doi.org/10.17221/49/2021-CJGPB

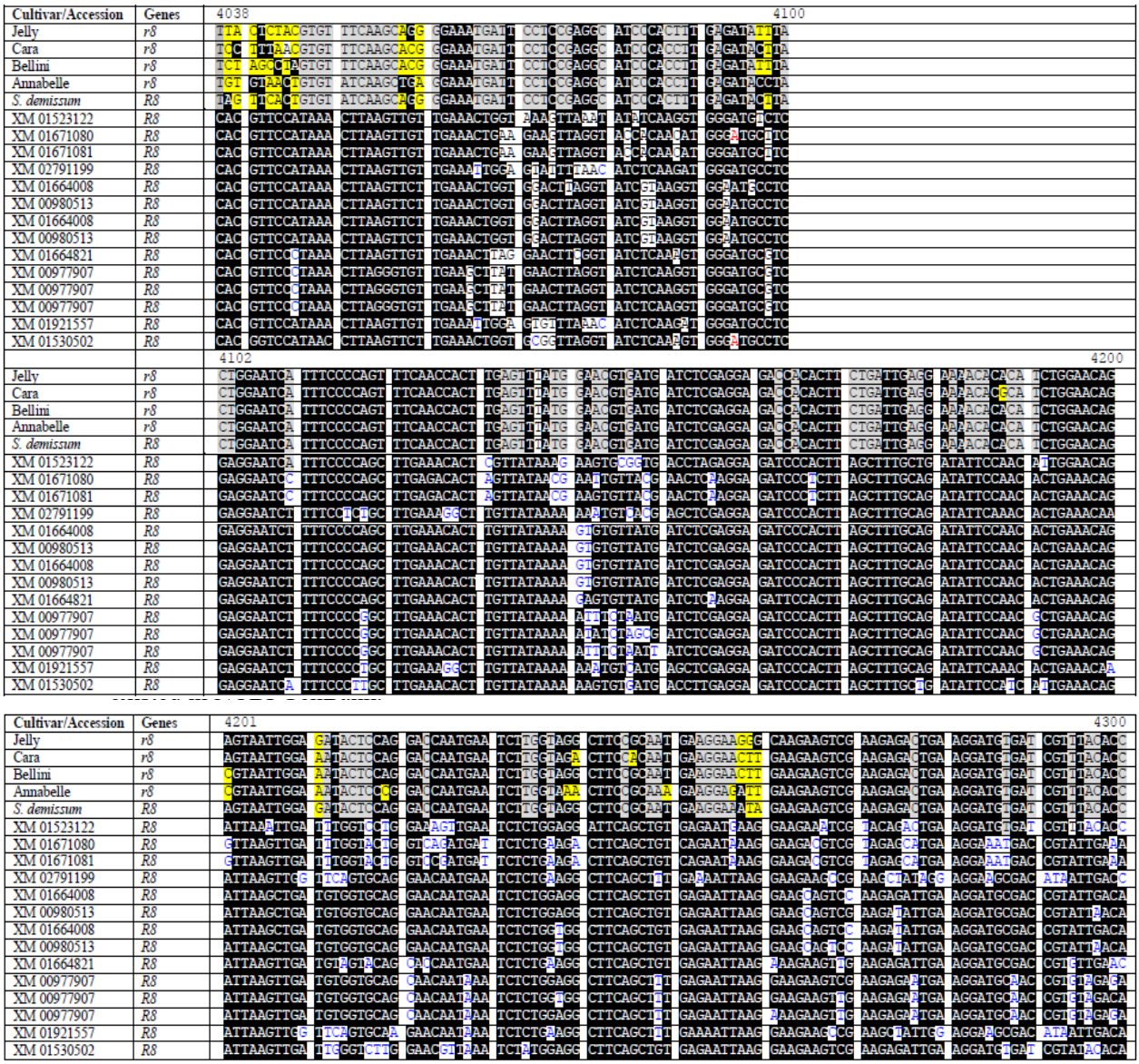

Figure 3. Sequence alignment of the *R8* and *r8* genes of the five potato genotypes, compared with the other accessions that exist in the NCBI GenBank

Table 4. Nucleotide variations and base-pair substitution in the five potato varieties and the NCBI accessions based on the two *R* gene similarities

Genes	Similarity of the five varieties with NCBI GenBank accessions																
	type of change	transversions								total	transitions				total	transition/ transversion	total = *N*
	exist in NCBI accessions as:	A		G		T		C			C	T	A	G			
	changed in five potato genotypes to:	C	T	C	T	A	G	A	G		T	C	G	A			
R3a/*ra3*		4	4	3	7	9	2	1	0	30	0	4	2	4	10	0.33	40
R8/*r8*		7	9	6	4	7	8	5	2	48	5	9	5	8	27	0.56	75
Total		11	13	9	11	16	10	6	2	78	5	13	7	12	37	0.89	115

https://doi.org/10.17221/49/2021-CJGPB

Table 5. Positional differences and base-pair substitutions in the nucleotide sequences among the five potato genotypes and the NCBI accessions based on the two *R* genes' similarity

Genes	Exist in NCBI accessions as:		A	A	A	A	A	G	G	G	G	G	G	C	C	C	C	C	T	T	T	T	T	T	T	Total changes
R3a	Jelly	changed to:	G	C				C		C				T					C	G	T					7
R3a	Cara		C	G				G		T				C					T	G	T					4
R3a	Bellini		C	G				G		T				C					T	G	C					5
r3a	Annabelle		G	C				C		C				T					C	C	T					7
r3a	*S. demissum*		C	C				C		C				T					C	G	T					7
	type (I)							1						1					1		1					4
	type (II)		1	1						1										1						4
r8	Jelly	changed to:	T	C	G	G	A	G	G	G	C	G	G	T	A	A	T	T	A	G	G	T	C	T	A	15
r8	Cara		C	C	C	T	A	G	A	A	T	T	C	A	A	C	C	T	A	G	A	T	T	T	A	16
r8	Bellini		C	A	C	T	A	G	G	G	A	T	C	C	T	T	T	T	A	G	A	G	C	T	A	16
r8	Annabelle		G	T	A	T	C	A	A	G	G	T	G	A	C	T	C	C	A	A	A	T	A	A	G	15
R8	*S. demissum*		A	T	A	T	A	G	G	G	T	A	G	A	C	G	C	T	A	G	G	T	C	T	A	12
	type (I)						1	1	1	1			1				1	1	1			1		1		10
	type (II)			1	1	1						1		1	1					1	1		1		1	10
	type (III)		1								1					1										3

two *R3a* and *R8* genes and the others recorded in the NCBI. Moreover, base-pair substitutions in the nucleotide sequences of the two tested genes among the five potato genotypes resistant and susceptible to LB are summarised in Table 5. The comparison of the nucleotide sequences of the two resistance genes revealed three types of base-pair substitutions. The first type (I): some nucleotides were transited from a purine to another purine base or pyrimidine to another pyrimidine base. For example, the guanine (G) base of the *R3a* gene in most NCBI accessions and the Bellini and Cara varieties under study was transited to the cytosine (C) base in Annabelle, Jelly and *S. demissum*. The second type (II): characterised by some nucleotides were transferred from a purine to a pyrimidine base or vice versa. For instance, the adenine (A) of the *R3a* gene in the NCBI potato clones was substituted into the cytosine (C) base in the Bellini, Cara and *S. demissum* genotypes and was replaced by guanine (G) in Annabelle and Jelly. The third type (III): contained both type (I) and type (II), revealed only in the *R8* gene, whereas the nucleotides of the five potato genotypes varied from each other and were also different from the nucleotides of the NCBI potato clones (Table 5). On the other hand, the highest total number of base-pair substitutions was scored in the *R8* gene in the Bellini and Cara varieties (16 substitutions), while the lowest one was recorded in the *R3a* gene of Cara (4 substitutions). Moreover, the total of base-pair substitutions was fourteen and three in both types (I and II) and III, respectively (Table 5). From the previous results, the presence of the positional differences and the base-pair substitutions in the nucleotide sequences of the two LB resistance alleles between the resistant genotypes (Jelly, Cara and *S. demissum*) and susceptible ones (Bellini and Annabelle) (Table 5) were observed.

Structural differences of two *R* genes between the resistant and susceptible potato genotypes. The coding DNA sequence (CDS) of the *R3a/r3a* and *R8/r8* loci in the five potato genotypes were encoded for 53–54 and 63–69 amino acid residues, respectively. The BLASTp search of the encoded R3a and R8 proteins in the five potato genotypes showed domain structures that are typical for R proteins from the family *Solanaceae* and other R proteins (i2, i2GA-SH23-3, i2C-5, I2C-2, RPP13-like protein 1, and CC-NBS-LRR) in the GenBank (Figure 4). On the other hand, the CDS of two genes differs in the resistant and susceptible potato genotypes to LB in the nucleotide sequences. These substitutions in the coding region led to differences in the amino acids ranging from 1 and 6 amino acids for the R3a and R8 proteins, respectively, as shown in Figure 4.

https://doi.org/10.17221/49/2021-CJGPB

Protein name	Varieties	Amino acid sequences
R3a	Jelly	PK**L**T**H**Q**L**ASSCHN*L**F**FY*VFSRVHSISKRFIKP**V**AHRYI**L**D**L**M**L**AYVS**L**HH**L**AQSEDR
R3a	Cara	YQ**L**T**D**Q**L**ASSCHN*L**L**FY*VFSRVHSIPKRFIKP**L**AHRYI**L**D**L**M**L**AYVS**L**HH**L**AQSEDR
R3a	Bellini	YH**L**T**S**L**L**VSSCHN*L**L**FYQVFSRVHSIPKRFIKP**L**AHRYI**L**D**L**M**L**AYVS**L**HH**L**AQSEDR
r3a	Annabelle	YH**L**TL*L**V**SSCHN*L**F**FY*VFSTVHSISKRFIKP**V**AHRYI**L**D**L**M**L**AYVS**L**HH**L**AQSEDR
r3a	*S. demissum*	PH**R**T**L**Q**L**ASSCHN*L**F**FY*VFSRVHSISKRFIKP**L**AHRYI**L**D**L**M**L**AYVS**L**HH**L**AQSEDR
r8	Jelly	**FTL**RVSSRGNDSSEASH**F**E**I****F**NCTSFNN**L**S**L**WNVISPRHHSSD*GKHTSSNWRYSR**L**GSSAMKE**G**
r8	Cara	**FL***RVSSTGNDSSEASH**L**E**I**L**N**CTSFNN**L**S**L**WNVISPRHHSSD*GKHASSNWKYSR**L**GSSTMKE**L**
r8	Bellini	**F*P**SVSSTGNDSSEASH**L**E**I****F**NCTSFNN**L**S**L**WNVISPRHHSSD*GKHTSRNWKYSR**L**GSSAMKE**L**
r8	Annabelle	**MCN**CVSS*GNDSSEASH**L**E**I****P**NCTSFNN**L**S**L**WNVISPRHHSSD*GKHTSRNWKYSR**L**GNSAKKE**I**
R8	*S. demissum*	**LVH**CVSSRGNDSSEASH**F**E**I**L**N**CTSFNN**L**S**L**WNVISPRHHSSD*GKHTSSNWRYSR**L**GSSAMKE**I**

Figure 4. Amino acid sequences architecture of the R3a, r3a, R8 and r8 proteins in the five potato genotypes under study

Phylogenetic relationship of the five potato genotypes based on the two *R* genes. The result of the phylogenetic tree of each potato genotype has the *R3a/r3a* and *R8/r8* gene target, based on its nucleotide base sequence, which was compared with known *R* genes from the family *Solanaceae* in the GenBank by the Neighbour-Joining method, using Geneious Pro (Ver. 4.5.4) software program (Figure 5). The dendrograms divided all the named GenBank potato accessions into various main discrete clusters, whereas the five potato genotypes under study formed a phylogenetically distinct cluster, separate from all the other named potato cultivars. Such observed results detected a large diversity to unique characteristics of the local resistant or susceptible potato varieties from all the other potato clones established around the world.

DISCUSSION

The potato (*S. tuberosum* L.) is the third most common food crop in the world after wheat and rice (Birch et al. 2012). Potato yield is threatened by late blight disease, caused by the oomycete *Phytophthora infestans* (Haverkort et al. 2009; Lenman et al. 2016). The present study is the first report to compare between dominant and recessive alleles of two genes *R3a/r3a* and *R8/r8* in some commercial potato varieties and wild species, as well as to report the presence of positional differences in the

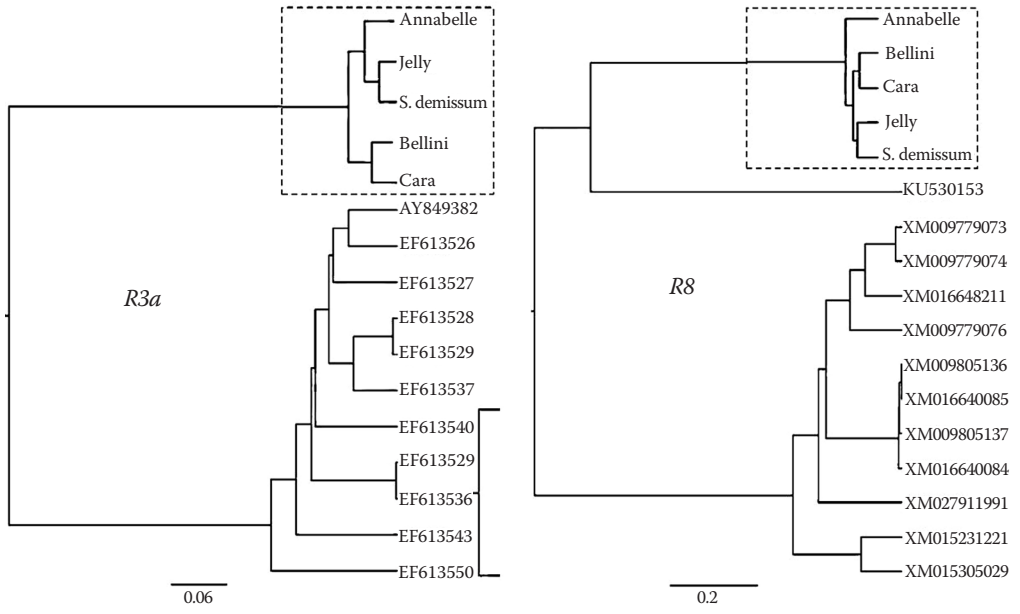

Figure 5. Phylogenetic relationships among the five potato genotypes under study, and the other GenBank related potato clones, based on the 194 and 220 bp of the *R3a* and *R8* genes, respectively

https://doi.org/10.17221/49/2021-CJGPB

nucleotide sequences and base-pair substitutions in the five potato genotypes under study.

The amplification of the *R3a* and *R8* genes in the five potato genotypes scored one amplicon of 194 and 220 bp, respectively. Up to now, twenty resistance (*R*) genes have been identified in *S. tuberosum* L. and wild species (*S. demissum*), e.g., *R1*, *R3a*, and *R3b* (Vossen et al. 2016; Jiang et al. 2018). These genes belong to the CC-NB-LRR class. In addition, they were derived from the wild *Solanum* species, such as *S. stoloniferum* and *S. papita*, (Vleeshouwers et al. 2008), *S. bulbocastanum* (van der Vossen et al. 2003, 2005), *S. venturii*, and *S. mochiquense* (Foster et al. 2009) and some commercial potato varieties (https://www.europotato.org). Yang et al. (2017) indicated a number of *R* genes resistance to LB from the wild species *Solanum demissum* have been introgressed into commercial potato cultivars by somatic hybridisation and sexual reproduction.

In this study, two fragments of *R3a* and *R8* were sequenced in three resistant varieties (Jelly, Cara and *S. demissum*), and two susceptible (Bellini and Annabelle) ones, and the percentage identity of the nucleotide sequences of the two genes ranged between (82–83%) and (86–87%), respectively, in comparison to the reference sequences in the nucleotide BLAST (http://blast.st-va.ncbi.nlm.nih.gov/Blast.cgi). The multiple sequence alignment showed several base-pair substitutions (transitions/transversions) between the resistant and susceptible potato genotypes to LB. In our study, we compared the base sequences of two genes between the resistant and susceptible genotypes. Little variations have been observed, from 1 to 6 frequencies, as shown in Table 3. Moreover, genetic polymorphisms among the resistant and susceptible genotypes were observed. In addition, the highest total number of base-pair substitutions was obtained in Bellini and Cara (*r8*) (16 bp), while the lowest one was 4 bp in Cara (*R3a*). On the other hand, conserved sequences were shared between the resistant and the susceptible genotypes, suggesting that they are not included in the resistance conferred by *R3a* and *R8*. These results were in agreement with Wang et al. (2015), who observed the presence of polymorphism (7-bp indel, insertion/deletion) between the dominant *Xa23* allele in the rice variety CBB23 and the recessive *xa23* allele in variety JG30.

In the current investigation, the genes of *R3a* and *R8* in the resistant and susceptible five potato clones encode the predicted 53–54 and 63–69 amino acid polypeptides, respectively. The amino acid sequences of the two genes were similar to NBS-LRR domains encoded by the *R* genes in the GenBank. In addition, the CDS of two fragments among the resistant and susceptible genotypes varied in amino acid sequences by insertion/deletion. These substitutions ranged from 9 and 13 amino acids for the R3a and R8 proteins, respectively. These results were confirmed by Bryan et al. (2000), who observed the presence of differences in the Pi-ta protein between resistant and susceptible rice varieties to rice blast by a single amino acid (serine instead of alanine at position 918), which they linked to the gene-for-gene specificity properties of the Pi-ta/AVR-Pita interaction.

Our data showed that the ratio of transitions to transversions of *R3a* and *R8* was 0.33 and 0.56, respectively. This was in contrast to other previous work, in which most of the reports used RNA-seq to scan the whole potato genome and genome-wide single nucleotide polymorphisms (SNPs), and no published reports focused on sequencing small fragments of LB resistance genes, as the current study did. For instance, Sevestre et al. (2020) indicated that the average rate of the transition (A/G or T/C) to transversion (A/C, T/A, C/G or G/T) ratio was 1.603. Both kinds of transition were revealed at an identical frequency at the genome-wide level. In contrast, the appearance of the transversion differed according to the nature of the substitution. The T/A transversions were the most frequent modification at the genome-scale, while the G/C transversions had the lowest frequency. However, the A/C and G/T transversions were intermediate. Other findings found that the ratio of transitions to transversions in *S. tuberosum* L. (1.48; Simko et al. 2006), *Beta vulgaris* (1.63; Schneider et al. 2001), and *Glycine max* (0.93; Zhu et al. 2003). Also, the ratio of transitions/transversions was 1 : 2 or 0.5. A clear bias toward transitions shows that each kind of transitional alteration is generated almost three times more compared with the transversional alteration. Simko et al. (2006) detected 1 145 sequence variants in a group of 47 potato clones, of which 95% were base-pair substitutions and the other 5% were indels. The ratio of transition to transversion was 1.5. Brown et al. (1982) stated that all the DNA sequences studied from any genome, the rate of transitions (T↔C and A↔G) is higher than the rate of transversions (T↔G, T↔A, C↔G, and C↔A).

In this work, a phylogenetic analysis was carried out depending on the multiple sequence alignment involving *R3a* and *R8* that constitute different clades with the *R* genes. These clades matched the *R* genes

https://doi.org/10.17221/49/2021-CJGPB

in the GenBank. In addition, we compared the differences in the genetic responses of two resistance genes, as a response to a *P. infestans* infection in resistant and susceptible potato genotypes, analysed the key loci in the metabolic pathway of resistance to the LB pathogen, and indicated the mechanisms of resistance against the pathogen. The suggested results in this work will create a solid base for the comprehension of strategies of host-pathogen interactions and supply a theoretical reference for the durable resistance in *S. tuberosum* L.

CONCLUSION

In summary, we have evaluated the genetic variability between the dominant and recessive alleles of two genes, *R3a/r3a* and *R8/r8*, in five potato genotypes resistant and susceptible to the LB disease. This analysis will help in understanding the relationship between the plant host resistance and the pathogen. A comparative study through nucleotide sequencing between dominant and recessive genes, in both the resistant and susceptible potato genotypes, was determined. From this data, the presence of minor variations at nucleotide levels were observed between the dominant and recessive alleles of two fragments, which led to changes in the amino acid sequence by insertion/deletion. These substitutions ranged from 9 and 13 amino acids for the R3a/r3a and R8/r8 proteins, respectively, among the tested potato genotypes.

REFERENCES

Birch P.R.J., Bryan G., Fenton B., Gilroy E.M., Hein I., Jones J.T. (2012): Crops that feed the world 8: Potato: are the trends of increased global production sustainable? Food Secure, 4: 477–508.

Brown W.M., Prager E.M., Wang A., Wilson A.C. (1982): Mitochondrial DNA sequences of primates, tempo and mode of evolution. Journal of Molecular Evolution, 18: 225–239.

Bryan G.T., Wu K.S., Farrall L., Jia Y., Hershey H.P., McAdams S.A., Faulk K.N., Donaldson G.K., Tarchini R., Valent B. (2000): A single amino acid difference distinguishes resistant and susceptible alleles of the rice blast resistance gene *Pi-ta*. The Plant Cell, 12: 2033–2045.

Foster S.J., Park T.H., Pel M.A., Brigneti G., Sliwka J., Jagger L., Van der Vossen E.A.G., Jones J.D.G. (2009): *Rpi-vnt1.1*, a *Tm-2* homolog from *Solanum venturii* confers resistance to potato late blight. Molecular Plant-Microbe Interactions, 22: 589–600.

Gebhardt C., Valkonen J.P. (2001): Organization of genes controlling disease resistance in the potato genome. Annual Review of Phytopathology, 39: 79–102.

Haverkort A.J., Struik P.C., Visser R.G.F., Jacobsen E. (2009): Applied biotechnology to combat late blight in potato caused by *Phytophthora infestans*. Potato Research, 52: 249–264.

Huang S.W., Vleeshouwers V.G.A.A., Werij J.S., Hutten R.C.B., Van Eck H.J., Visser R.G.F., Jacobsen E. (2004): The *R3* resistance to *P. infestans* in potato is conferred by two closely linked *R* genes with distinct specificities. Molecular Plant-Microbe Interactions, 17: 428–435.

Huang S.W., Van der Vossen E.A.G., Kuang H., Vleeshouwers V.G., Zhang N. (2005): Comparative genomics enabled the cloning of the *R3a* late blight resistance gene in potato. The Plant Journal, 42: 251–261.

Jiang R., Li J., Tian Z., Du J., Armstrong M., Baker K., Lim J.T., Vossen J.H. (2018): Potato late blight field resistance from QTL dPI09c is conferred by the NB-LRR gene *R8*. Journal of Experimental Botany, 69: 1545–1555.

Kochetov A.V., Glagoleva A.Y., Strygina K.V., Khlestkina E.K., Gerasimova S.V., Ibragimova S.M., Shatskaya N.V., Vasilyev G.V., Afonnikov D.A., Shmakov N.A., Antonova O.Y., Gavrilenko T.A., Alpatyeva N.V., Khiutti A., Afanasenko O.S. (2017): Differential expression of NBS-LRR-encoding genes in the root transcriptomes of two *S. phureja* genotypes with contrasting resistance to *Globodera rostochiensis*. BMC Plant Biology, 251: 41–50.

Lenman M., Ali A., Mühlenbock P., Nilsson C.U., Liljeroth E., Champouret N. (2016): Effector-driven marker development and cloning of resistance genes against *P. infestans* in potato breeding clone SW93-1015. Theoretical and Applied Genetics, 129: 105–115.

Li G.C., Huang S.W., Guo X., Li Y., Yang Y., Guo Z. (2011): Cloning and characterization of *R3b*; members of the *R3* superfamily of late blight resistance genes show sequence and functional divergence. Molecular Plant-Microbe Interactions, 24: 1132–1142.

Mahfouze H.A., Ahmed H.Z., El-Sayed O.E. (2021): Gene expression of pathogenesis-related proteins and isozymes in potato varieties resistant and susceptible to late blight disease. International Journal of Agriculture and Biology, 26: 490–498.

Petit-Houdenot Y., Fudal I. (2017): Complex interactions between fungal avirulence genes and their corresponding plant resistance genes and consequences for disease resistance management. Frontiers of Plant Science, 8: 1–8.

Quirin E.A., Mann H., Meyer R.S., Traini A., Chiusano M.L., Litt A., Bradeen J.M. (2012): Evolutionary meta-analysis of Solanaceous resistance gene and *Solanum* resistance gene analog sequences and a practical framework for

https://doi.org/10.17221/49/2021-CJGPB

cross-species comparisons. Molecular Plant-Microbe Interactions, 5: 603–612.

Schneider K., Weisshaar B., Borchardt D.C., Salamini F. (2001): SNP frequency and allelic haplotype structure of *Beta vulgaris* expressed genes. Molecular Breeding, 8: 63–74.

Sevestre F., Facon M., Wattebled F., Szydlowski N. (2020): Facilitating gene editing in potato: A single-nucleotide polymorphism (SNP) map of the *Solanum tuberosum* L. cv. Desiree genome. Scientific Reports, 10: 2045.

Shao Z.Q., Wang B., Chen J.Q. (2016): Tracking ancestral lineages and recent expansions of *NBS-LRR* genes in angiosperms. Plant Signal and Behavior, 11: e1197470.

Simko I., Haynes K.G., Jones R.W. (2006): Assessment of linkage disequilibrium in potato genome with single nucleotide polymorphism markers. Genetics, 173: 2237–2245.

van der Vossen E.A.G., Sikkema A., Hekkert B.T.L., Gros J., Stevens P., Muskens M., Wouters D., Pereira A., Stiekema W., Allefs S. (2003): An ancient *R* gene from the wild potato species *Solanum bulbocastanum* confers broad-spectrum resistance to *Phytophthora infestas* in cultivated potato and tomato. Plant Journal, 36: 867–882.

van der Vossen E.A.G., Gros J., Sikkema A., Muskens M., Wouters D., Wolters P., Perira A., Allefs S. (2005): The *Rpi-blb2* gene from *Solanum bulbocastanum* is an *Mi-1* gene homolog conferring broad-spectrum late blight resistance in potato. Plant Journal, 44: 208–222.

Vleeshouwers V.G.A.A., Rietman H., Krenek P., Champouret N., Young C., Oh S.K., Wang M., Bouwmeester K., Vosman B., Visser R.G.F., Jacobsen E., Govers F., Kamoun S., Van der Vossen E.A.G. (2008): Effector genomics accelerates discovery and functional profiling of potato disease resistance and *Phytophthora infestans* avirulence genes. PLoS ONE, 3: e2875.

Vossen J.H., Van Arke G., Bergervoet M., Jo K.R., Jacobsen E., Visser R.G.F. (2016): The *S. demissum R8* late blight resistance gene is an *Sw-5* homologue that has been deployed worldwide in late blight resistant varieties. Theoretical and Applied Genetics, 129: 1785–1796.

Wang C., Zhang X., Fan Y., Gao Y., Zhu Q., Zheng C., Qin T., Li Y., Che J., Zhang M., Yang B., Liu Y., Zhao K. (2015): XA23 is an executor R protein and confers broad-spectrum disease resistance in rice. Molecular Plant, 8: 290–302.

Yang L., Wang D., Xu Y., Zhao H., Wang L., Cao X., Chen Y., Chen Q. (2017): A new resistance gene against potato late blight originating from *Solanum pinnatisectum* located on potato chromosome 7. Frontiers in Plant Science, 8: 1729.

Zhu Y.L., Song Q.J., Hyten D.L., Van Tassell C.P., Matukumalli L.K. (2003): Single-nucleotide polymorphisms in soybean. Genetics, 163: 1123–1134.

Received: May 31, 2021
Accepted: October 11, 2021
Published online: November 4, 2021